Next step? The switch from stool culture to PCR

David Wild

September 2018—The advantages of moving from stool culture to a molecular platform are many: faster time to results, more accurate pathogen identification, a savings of space and staff time. For Jose Alexander, MD, D(ABMM), SM, MB(ASCP), and colleagues at Florida Hospital Orlando, another plus is being able to adhere to the Infectious Diseases Society of America guideline suggestion that labs use a diagnostic approach that can distinguish O157 from non-O157 *E. coli* and Shiga toxin 1 from Shiga toxin 2 *E. coli*.

Dr. Alexander, a medical and public health microbiologist and the hospital's director of clinical microbiology, shared the story, in a webinar this spring and in a recent interview, of how his laboratory made the decision to switch to PCR.

"Moving from stool culture to a molecular platform seems to be the next big step for many laboratories, not only for the improvement of the technique for detection of pathogens and the benefits to the patient but also for the benefits to the laboratory itself," he said in the webinar, which was hosted by CAP TODAY and made possible by a special educational grant from Luminex.

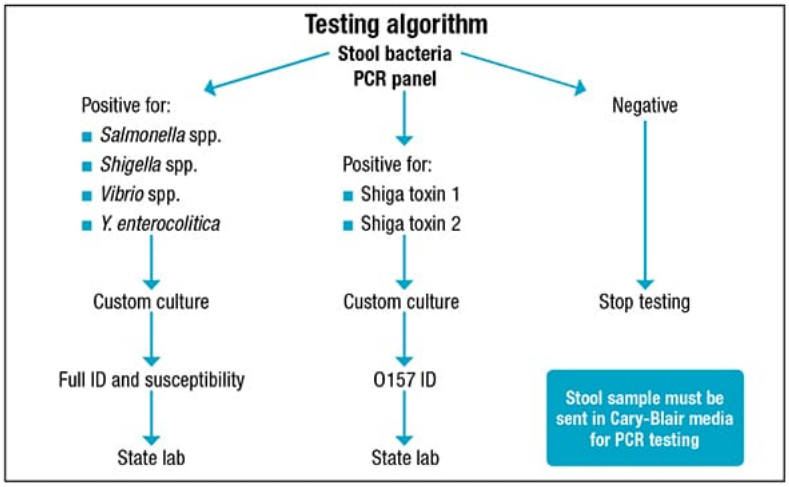
One of the first things Dr. Alexander and his colleagues at Florida Hospital Orlando considered when weighing a switch was the department's stool culture volume and the cost implications. The Florida Hospital system has a 24/7 central microbiology department staffed by 42 full-time medical technologists and technicians for its 2,400 beds across seven hospitals and multiple long-term and nursing home facilities. By 2017, when the department made its case for molecular testing in place of culture, 780,000 tests were performed in the department, of which 7,200 were stool cultures.

"One of the most important steps in making the case for a switch to PCR and selecting the right panel for us was to document the types of organisms we had been screening for through stool cultures," Dr. Alexander said.

In 2016, the microbiology department cultured 6,800 stool samples, two-thirds of which were for outpatients, and found the most prevalent pathogen was *Salmonella* spp., with 163 cases, followed by 82 cases of *Campylobacter* spp. and 28 cases of *Shigella* spp. Other less common pathogens isolated included four cases of *Escherichia coli* O157, two cases of *Yersinia enterocolitica*, four cases of *Plesiomonas shigelloides*, five cases of Shiga toxin 1-producing *E. coli*, and two cases of *Shiga* toxin 2-producing *E. coli*.

Although this pathogen distribution matched what the Centers for Disease Control and Prevention's Foodborne Diseases Active Surveillance Network reported in recent years, Dr. Alexander said, "we still felt like stool cultures were not actually detecting the amount of organisms that are circulating in our population."

Testing by PCR would mean redistributing the benches and staff, a 24-hour turnaround time, greater sensitivity, and improved in-house viral detection. "We can also have more space in the ambient incubator," he said. It also meant having to purchase less special media and being able to screen routinely for *Vibrio* and *Yersinia*, which are now a separate order. "But the most important advantage is we can screen for Shiga toxin genes for detecting non-O157 and also non-toxin-producing Shiga toxin *E. coli*." Routine culture will not detect many non-O157 or low-level



producing or non-producing Shiga toxin *E. coli*.

Switching to PCR meant, too, that Florida Hospital would fall in line with the 2017 Infectious Diseases Society of America's Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea, which encourage use of "culture-independent methods" of stool testing for possible bacterial or parasitic causes as well as possible *C. difficile* infection.

When Dr. Alexander and colleagues calculated the cost of a culture, including the special media, GN broth, Shiga toxin EIA, and Vitek ID cards, they "got a big surprise," finding that a single stool culture cost nearly $28. When they factored in the cost of operator time—five minutes for setup and 30 minutes of hands-on time per culture over five days—the cost rose to almost $53 on average.

"With PCR, the hands-on time is down from 30 minutes to less than five minutes per sample, and we can remove a bench," he said.

Dr. Alexander and his colleagues decided they would transition to PCR stool testing and deliberated between two platforms: the BioFire FilmArray and the Luminex Verigene Enteric Pathogens test. They chose the latter, in part because the platform was already in-house.

"One of the advantages of the Verigene for us was that it included many of the targets indicated in the IDSA guidelines and we could create custom bacterial or viral panels," Dr. Alexander said. "The BioFire has multiple other targets and has a very interesting group of targets for parasites, but it also includes *C. difficile*, which we were already testing for using a separate molecular platform and a completely different protocol. Keeping *C. difficile* separate made implementation a bit easier."

He explains: "The IDSA *C. difficile* guideline came out at a good moment for us." It says when there are no pre-agreed institutional criteria for patient stool submission, the "double step" approach is best—GDH plus toxin, GDH plus toxin arbitrated by nucleic acid amplification testing, or NAAT plus toxin. "We use GDH and toxin, and discrepancies are solved using a PCR platform," one that is different from Verigene. "And this is something we already implement and we continue to be performing in that direction."

While the Verigene test does not identify parasites, the clinical manifestation and epidemiological background of parasitic infections is "quite different from bacterial and viral causes," he said. With no parasite panel to include, he added, "we are going to deal with the parasite in a completely different scenario."

They asked themselves whether they needed a panel to detect *Plesiomonas shigelloides* and *Aeromonas*—which the Verigene test does not include—and decided they did not, since the health system isolated only two *P. shigelloides* and no *Aeromonas* in 2016 using stool culture. "Although those two organisms were not a concern for us and not necessary to include in a molecular panel, we did decide to offer a custom stool culture for these organisms in case the microbiology department wanted to test for these," Dr. Alexander said.

Once they determined the Verigene system best suited their needs for enteric pathogen testing, Dr. Alexander and his colleagues validated the sensitivity and specificity of the test using 58 frozen stool samples they had previously cultured. Ten were known negative and 48 were known positive and included *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Vibrio*, *Yersinia enterocolitica*, Shiga toxin 1 and 2 *E. coli*, as well as rotavirus and norovirus. Dr. Alexander and his team found the panel was 100 percent sensitive and 100 percent specific: "All the targets were detected as suspected."

"Worth noting is that the custom Verigene multiplex panel we built includes nine different targets, but the samples we used only tested positive for the single target we tested for," Dr. Alexander said. "So that means every time you run the sample, you have one positive target detected but you also have eight negative targets. This is something to consider in your validation protocol."

They also tested for reproducibility by running at least five samples twice by the same operator and for precision by running at least five samples twice by two different operators. "One hundred percent reproducibility and

precision were obtained," he says.

Dr. Alexander and his colleagues ultimately created four custom panels: one for bacteria (*Salmonella, Shigella, Vibrio*, and *Y. enterocolitica* as well as Shiga toxin 1 and 2 [enterohemorrhagic *E. coli*, or EHEC] and *Campylobacter*), one for viruses (including rotavirus and norovirus), and two separate panels testing for rotavirus and norovirus as single targets.

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"We decided to offer the norovirus and rotavirus tests separately as single targets for our infection prevention team who might want to do a contact screening for patients with known norovirus or rotavirus, or for patients with diarrhea after more than three days of hospitalization who have been in contact with, or in close proximity to, a patient with norovirus or rotavirus," Dr. Alexander explained. In this way, "the cost of the test for single target is less than for the entire panel."

Depending on the PCR finding, Florida Hospital providers will, in collaboration with the microbiology laboratory, be able to follow up with a custom culture to more fully identify the organism and test for susceptibility. (See "Testing algorithm," page 52.)

"And we are planning on submitting culture results to the state lab, which is very important, because moving from routine stool culturing to a molecular platform without collaborating with the public health department can have a negative impact. It's very important that you communicate with them and they know you plan to move to a different platform."

To provide clinical guidance while pathogens are undergoing susceptibility testing, the laboratory creates an enteric pathogen antibiogram every year. "Having the background data from the antibiogram can help the physician decide whether or not to treat the patient and which antibiotic to use," he said. "I think it's a good idea to go back into your system and develop a specific antibiogram for those organisms included in the panel."

Dr. Alexander used the Verigine instrument when he worked at Marin General Hospital in Greenbrae, Calif. He and his colleagues there compared the prevalence of enteric pathogens identified from stool cultures in 2014 and by PCR testing in 2015 after they switched to the molecular platform. They had tested inpatients and outpatients with diarrhea and found PCR identified notably more of several pathogens.

"A striking thing we found was that the number of *Campylobacter*-positive isolates almost quadrupled, from 14 to 55 with PCR," he said. "More *Shigella* cases were also identified [seven compared with two], and we detected the first and only *Y. enterocolitica* at the institution."

The number of *Vibrio*-positive samples also increased from two to six, but "the most interesting data came for results from Shiga toxin *E. coli*," Dr. Alexander said. PCR detected 16 isolates with Shiga toxin genes, including four O157 organisms, six O26 organisms, and six Shiga toxin-producing organisms that did not react with antigen O during stool testing. In addition, nine organisms with PCR-detected Shiga toxin genes did not produce Shiga toxin despite having the gene.

"If you're only relying on culture-based methods in your facility right now, you're only identifying O157 and Shiga toxin *E. coli* that produce toxin, which means you might be reporting false-negatives," he said.

There are public health implications. "We've been told for many years that O157 is the most common type of *E. coli* in the United States, but the problem is that if a clinical lab is only screening for O157 or Shiga toxin-producing organisms, only those data will be reported to the state, so the data could be skewed," he said.

Patients in the Marin General ER presenting with diarrhea previously often went home while a stool culture was in progress, he said, whereas with PCR many of the patients received results while still in the ER. "That means some patients can go home with a targeted antibiotic if required."

Providers need to understand the advantages and limitations of PCR and have results interpreted for them as much as possible, Dr. Alexander said. "Make sure that in the description of the test results you indicate all the species that were screened for in the panel."

And avoid reporting only "Shiga toxin."

"Simply writing that you've found Shiga toxin 1 or 2 is probably not the most useful way to report your findings. If the patient has enterohemorrhagic *E. coli*, we emphasize that, as well as mention the type of Shiga toxin found."

Providers should also be aware of what types of tests can be performed for stool and the laboratory requirements for specimen collection and transport, he added.

"And let physicians know what kind of testing will be done after a specific pathogen is found. Make it clear that after a positive *Campylobacter* finding, you will not be performing additional tests, or that when a specimen is positive for Shiga toxin, it will be sent to the public health department for further testing."

He offers the following common questions:

- *How many samples must a lab accept, and when is it appropriate to retest?* "At Florida Hospital we plan to accept one sample per person to be submitted to the laboratory. We are still working with our infection prevention and infectious disease groups to determine when it would be appropriate to receive a second sample for retesting."
- *Can PCR for enteric pathogens be performed stat?* "This is probably the most common question you would receive from emergency department providers. When they find out you have a rapid identification method for identifying enteric pathogens and the results can be back while the patient is still in the ED, they'll be asking about stat testing. We will have 24-hour turnaround but plan to go down to six hours or maybe less."

- *Should the targets be offered separately or by group?* "We don't think the bacterial targets should be offered separately. They should be grouped just as we do with cultures. The viral targets can be offered along with the bacterial targets and/or as a single target in case norovirus or rotavirus is the suspected agent," to decrease the cost of the test.
- *Should bacterial, viral, and parasite targets be performed all at once?* "Parasites have different conditions for epidemiological purposes, which is something your infection prevention and infectious disease groups need to consider, particularly when working with your outpatient providers. Parasite testing volume is mainly from outpatient settings, since some of the infections can be subclinical and/or chronic." Infection prevention and ID physicians, along with pharmacy, should always be involved and able to provide feedback and recommendations, he says.
- *What do you do with a positive result?* "I think the best thing to do is to work with the public health department and enter information about susceptibility if you have previous data, for empirical treatment or if a physician needs that information. At the same time, you want to be able to identify the pathogen and submit that information to the public health department and as part of your quality assurance process for the system."
- *Would it be necessary to continue with a limited stool culture?* "We will not be offering physicians the option of single or standalone stool culture. However, if a provider believes a culture is needed, they will be able to discuss the case with the laboratory and we may proceed with a culture if it's appropriate."

David Wild is a writer in Toronto.